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COMPARATIVE MORPHOLOGY OF EXPERIMENTAL TUBERCULOSIS AND BRUCELLOSIS IN GUINEA PIGS

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The morphology of experimental brucellosis and tuberculosis in guinea pigs has been given detailed study (for literature see Page 1 on tuberculosis and Ariyel' on brucellosis). A majority of authors consider Brucella granulomata in guinea pigs very similar to tubercles (Smith and Fabian, Klimmer and Haupt, Lotze, Seiphart, and others). A similarity with tubercles is observed also in Brucella granulomata in man (Roessle, Nicod), but in contrast to tubercles, scarring (Nicod) and necrosis of cellular elements in granulomata (Loeffler and Albertini, Nicod) are not observed in brucellosis in man. This information exhausts the literature data on the comparative morphology of tuberculosis and brucellosis. The comparative morphology of these diseases has not been studied in experimental conditions.

When there is doubt about brucellosis, we resort to infecting guinea pigs with the test material. This method has been used for differential diagnosis of brucellosis in man in clinical conditions (Hartley and Jordan), in veterinary practice (Feldmann and Olson), and in autopsy (Rothmann).

This method has great practical value, since the guinea pig is very sensitive to all species of Brucella. Therefore, we made a comparative study of the morphological changes in experimental tuberculosis and brucellosis in guinea pigs in order to obtain more detailed data on the comparative morphology of these diseases.

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Material and Method

Guinea pigs were injected subcutaneously in the anterior right thigh with tubercle bacillus and Brucella cultures. The stimulator was lymphogenously diffused in the area of the inguinal lymphatic nodules. In this manner identical and anatomically defined primary complexes were obtained in both cases.

Our experiments were divided into two groups. The first group was composed of 18 guinea pigs which were injected with a dose of 20,000 microbes of a virulent species of Maltese micrococci (M-85). (This group of animals were used in one of a series previous experiments, the results of which were published earlier in studies dedicated to the pathological morphology of experimental brucellosis, using the same method. The experiments on the infection of guinea pigs with Brucella were conducted in the brucellosis section of the Department of Pathogenic Infections of VIEM.)

The second group was composed of 15 guinea pigs which were injected with a virulent species of tubercle bacillus. Then, 0.3 cc of a culture dilution in physiological salt solution (1 mg of culture in 1 cc of physiological salt solution) were injected into the guinea pigs.

The guinea pigs usually died 3½ to 4 months after injection with tubercle bacillus. The animals in both experimental groups were studied at various intervals after injection.

The skin of the injected area in the right anterior thigh, regional (inguinal) lymphatic nodules, lungs, liver, spleen, appendia testis, and 5-6 lymphatic nodules which were not in the area where the culture was injected, were obtained for histological study.

In addition to the usual methods, tissues were stained by the Foote /Russian "fut" may be Foote, Furth/ method and for tubercle bacillus.

Macroscopic Changes

About 2-3 days after injection with brucella, a slight elevation of the skin and subcutaneous cellular tissues develop in the area of the injection site. No change whatsoever could be discovered in this area after a week. Meanwhile, the regional lymphatic nodules became enlarged and acute hemorrhagic inflammation in the subcutaneous cellular tissue developed in the surrounding area, about the thigh, and in the anterior abdominal wall.

In tuberculosis acute degeneration develops both in the area of the injection site and in the regional lymphatic nodules. A rapidly progressive abscess with walls covered with a caseous degeneration is formed in the injection site. The abscess ruptures and approximately 3 weeks after the injection an ulcer is formed, the base of which is covered with pus and caseous masses. The regional lymphatic nodules become considerably enlarged, reaching a greater size than in guinea pigs infected with brucellosis. They attain the size of a pea or bean after approximately one month. The nodules are first hard, caseous, and of a yellow color in cross-section; in the later stages the caseous masses become soft. The subcutaneous cellular tissue about the lymphatic nodules is somewhat swollen and compressed. In contrast to brucellosis, the inflammatory changes in the tissue around the lymphatic nodules never assume a hemorrhagic character.

In contrast to general brucellosis where no macroscopic changes are noticed in the internal organs, a few grey miliary nodules are discovered after approximately 10-14 days in general tuberculosis. Their number in-

creases rather rapidly, especially in the liver and spleen. They increase in size and begin to protrude clearly in the form of many relatively large yellowish nodules. Accompanying this, large yellow-white nidi of a type of infarcts appear after 1½-2 months. Also, caseous nodules colored a bright yellow with bile are observed in the liver. A bilious fissure can sometimes be seen in their center. Together with an acutely enlarged liver and spleen, a thickened, hard yellow-white epiploon is discovered upon dissection of the abdominal cavity. Changes in the lungs develop somewhat slower than in the liver and spleen. The miliary nodules gradually increase in size. They first take the form of thick, white, soft-centered caseous nodules reaching the size of a pea. In later stages, thick yellowish-white nidi are sometimes noticed also in the appendix testis.

Thus, in brucellosis, the stimulator, while leaving almost no changes in the inoculation site, is lymphogenously disseminated and causes acute morphological changes in the regional lymphatic nodules (incomplete primary complex).

On the other hand, in tuberculosis, changes take place both in the injection site of the stimulator and in the regional lymphatic nodules (complete primary complex).

In general brucellosis no changes are visible to the naked eye, while in general tuberculosis there are definite macroscopic changes.

Microscopic Changes

About 24 hours after an injection of Brucella, edema, swelling of the fibroblasts, and infiltration of leukocytes and polyblasts are observed in the skin and subcutaneous cellular tissue. These phenomena soon undergo a reverse development; no changes were observed 7 days later in the site injected with cultural specimens.

In injection with tubercle bacillus, a nonspecific inflammatory process also develops in the onset in the subcutaneous cellular tissue, but in contrast to brucellosis it has a progressive character. An abscess containing a considerable quantity of bacilli is formed in the injected site after 3 days. A nonspecific granulation tissue containing fibroblasts, polyblasts, and leukocytes is seen on the periphery of the abscess. Singular and rather large epithelioid cells can also be noticed after 8 days and gradually increase in number. Giant cells of the Langhans type appear among them after 2 weeks. Only singular bacilli are discovered in the protoplasm of the epithelioid cells; on the other hand, the number of bacilli is very great in the abscess forming the central part of the nidus. The cellular tissue about the abscess becomes swollen. Nidi with caseous and necrotic degeneration are discovered infiltrated with disintegrated leukocytes in the specific granulation tissue after 2 weeks. The caseous masses contain a large number of bacilli. The abscess gradually increases and the surrounding epithelial cells become necrotic. By this time a serous liquid and leukocytes appear in the center of the nidus; the caseous masses gradually become soft, the abscess ruptures and an ulcer is formed (after 1½ months). At the base of this ulcer, homogeneous and caseous masses and epithelioid tubercles separated from each other by friable layers of connective tissue are seen. Part of the tubercles in the center are necrotic and an accumulation of lymphocytes and plasmatic cells is seen in their periphery. Lumps of lime appear in the necrotic masses of the bottom of the ulcer in the later stages (4 months).

The walls of the arteries situated among the cells of the granulation tissue are often homogenized. As a result of the proliferation of the

cellular elements in the internal layer, the lumen of part of them is constricted or obliterated.

A considerable accumulation of eosinophilic leukocytes is observed in the regional lymphatic nodules after an injection of Brucella. Later, nidus accumulations of macrophages which completely replace a large part of the follicles are formed in the nodules due to hyperplasia of reticulo-endothelial cells. Singular multinucleate giant cells are also found among the macrophages. Their number later increases. Necrosis of the cellular elements in the granulation tissue is observed only in singular cases of brucellosis.

A dilation of the sinuses is observed in the regional lymphatic nodules 24 hours after injection with tubercle bacillus. Macrophages and lymphocytes appear in the sinuses, while the leukocyte count in follicles decreases. Together with this, the nodules become infiltrated with a considerable quantity of leukocytes. After 3 days swollen reticular cells are observed in the follicular vacuoles. They form small nidi which increase in size considerably faster than the nidi formed by the macrophages in brucellosis. After 10 days these nidi are filled with large compact cells forming a variety of syncytia in places. They are infiltrated with a large quantity of partially disintegrated leukocytes which form small abscesses in some places. The nidi develop and reach the size of capsules. These capsules are thickened in these sections; fibroblasts and a few polyblasts are seen among the fibrous tissues.

The sinuses are greatly dilated and filled with macrophages and lymphocytes. Irregularly defined nidi of caseous necrosis containing a large quantity of tubercle bacilli are observed in the nidi formed by the elements of hyperplastic reticular stroma after 2 weeks. Cellular elements on the periphery of some of the nidi gradually take on the character of epithelioid cells. Their protoplasm contains singular tubercle bacillus. Singular giant cells of the Langhans type gradually appear among the epithelioid cells. The caseous masses contain a large number of chromatinic clumps which are frequently arranged in concentric rings. The caseous masses become soft in the later stages. A serous liquid containing a large number of leukocytes is accumulated in them. In connection with this, abscesses containing spherical granulation tissue are formed in the regional lymphatic nodules after 3-3½ months. There is a considerable quantity of connective tissue fibers among the cells of the elements of the later (epithelioid cells and fibrocytes). The capsules of the lymphatic nodules are also greatly thickened.

In separate (not regional to the site of injection) lymphatic nodules there is observed a decrease in the lymphocyte count in the periphery of the reactive centers in the primary stages after injection of Brucella; a considerable number of plasma cells appear in the brain tissues, and symptoms of catarrh develop in the sinuses. Small nidi composed of macrophages, among which there are multinucleate giant cells, are observed in the follicles after 10 days. These nidi grow rather rapidly and unite in places.

In tuberculosis, together with an infiltration of the lymphatic nodules by leukocytes, the lymphocyte count in the follicles decreases, and macrophages and lymphocytes appear in the dilated sinuses. Just as in brucellosis, a large number of plasma cells are discovered in the brain tissues. All of these changes progress rather slowly, and nidi of epithelioid cells appear in separate follicles only after 3-4 weeks. They gradually grow and their central sections undergo caseous necrosis. The caseous masses first contain a large quantity of nuclear disintegration elements and in the later stages become homogenized. A considerable quantity of collagenous fibers

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are formed on the periphery of the necrotic nidi between the cellular elements of granulation tissue. The described changes have a predominantly nidal character, and do not reach that degree of distribution which was noticed in the lymphatic nodules regional to the injection site of the cultural specimens.

Hypertrophy and a gradually increasing hyperplasia development of Kupffer's cells are observed in the liver during the early stage after injection with Brucella. Accumulations of polyblastic cells with polymorphous dark nuclei appear later in the capillaries. Small granulomata containing polyblasts and epithelioid cells can be seen inside the hepatic lobules as well as in the adventitia of the small veins. The number of such granulomata increases comparatively fast. A large number of small granulomata containing epithelioid and giant cells are observed in the liver after 1-1½ months. Part of the granulomata are situated in the periportal layers, and sometimes they completely replace the latter.

Just as in brucellosis, a slight hypertrophy and moderate proliferation of Kupffer's cells is observed in the earlier stage after injection with tubercle bacillus. Singular nodules containing polyblasts, among which epithelioid cells soon appear, are formed within the lobules after 5-8 days. Such nodules grow very rapidly and after 2 weeks reach a relatively large size in comparison with granulomata observed in brucellosis. A few tubercle bacilli are observed among the cellular elements in the granulation tissue after 1½-2 months. Some of the granulomata are situated in the periportal layers. The lymphatic vessels of the latter are greatly expanded. Very fine collagenous fibers, the quantity of which rapidly increases, are seen among the cellular elements of the granulation tissue. After 2 months the liver is perforated with wide strands of connective tissue, among the fibers of which are seen singular epithelioid and giant cells and a large quantity of false bile passages. The cells in these strands gradually disappear, while the quantity of fibers constantly increases. Only proliferating false bile passages and singular polyblasts are observed between the fibers in the later stages. In such places where the connective tissue strands adjoin the lobules of the liver, transitional forms from the compressed and atrophied hepatic cells to the so-called false bile passages can be seen.

Very acute changes in the vessels of the liver are observed comparatively early. The wall of the hepatic artery becomes dilated and is homogenized; the endothelium also becomes enlarged. Changes in the artery are of an extensive character.

Sharper changes are observed in the branches of the portal vein in the hepatic parenchyma. In the portal vein, proliferation of the cellular elements of the intima begins comparatively early (1½ months), and then granulomas without a specific structure are formed adjacent to the wall. A large part of the vein of the periportal layers is completely obliterated, and the clearance of the larger veins is sharply contracted. In some cases, with regard to adventitia, specific granulation tissue which sometimes becomes necrotic develops in the clearance of the vein.

In conjunction with the effect in the branches of the portal vein, extensive necroses of the type of anemic infarcts are formed in the liver. Both the parenchyma of the liver and the specific granulation tissue, rich with collagenous fibers, undergo necrosis. On the periphery of the infarcts there is sometimes observed a demarcation border of leukocytes which fill out the greatly dilated capillaries adjoining the necrotic nidi.

Together with a slight leukocytic infiltration of the pulp, the lymphocyte count gradually decreases in the follicles, and giant cells of a macrophage character appear in the spleen in the early stages after injection with Brucella. They form in the pulp as well as in a large part of the follicles

of the nidus, reaching considerable dimensions after approximately 3 weeks. Singular giant cells and leukocytes are seen among the macrophages. After 1½ months the border between the follicles and the pulp of the spleen disappears because of the fusion of these nidi. Newly-formed cells separate the reticular fibers of the stroma of the organ; however, new formation of connective tissue fibers in the spleen as well as in other organs is never observed in brucellosis.

A very acute erythro-pigmentophagia, together with leukocytic infiltration in the pulp of the spleen, is seen during the early stages following the injection of tubercle bacilli. The lymphocyte count in the follicles gradually decreases. Small macrophagic nidi appear on the periphery of the latter after approximately 10 days. They increase in size rapidly and after approximately 3 weeks replace separate follicles which are situated in the adjacent parts of the pulp. Giant cells and numerous leukocytes are noticed among the macrophages which are gradually acquiring the character of epithelioid cells. The granulomata increase in size and their foci undergo caseous necrosis, which is not observed in brucellosis. A few tubercle bacilli are observed in the protoplasm of the epithelioid cells and in the necrotic masses. The necrotic masses are homogenized, and lime appears in them in the later stages. Sometimes a serous liquid develops in the caseous masses and there is an accumulation of leukocytes which is then followed by a softening of these masses. Collagenous fibers appear relatively early among the cellular elements of the granulation tissue and increase later. On the periphery of a singular necrotic nidus a wide collagenous capsule is formed. In such cases where the necrotic masses are adjacent to a capsule, the latter becomes greatly swollen.

The veins and sinuses of the spleen become dilated comparatively early. They contain a considerable quantity of serous liquid and leukocytes. The granulation tissue situated between the dilated sinuses is often swollen; a considerable accumulation of plasmatic cells is seen in places between the sinuses. Obturating and wall thrombi are formed in parts of the dilated sinuses, while in other sinuses, specific granulomata appear. Nonspecific obliterating endarteritis is observed in many arteries. Anemic infarcts are formed in the spleen and in the liver in conjunction with infection of the vessels.

Proliferation of the connective tissue elements in adventitia of small veins and interalveolar partitions are noticed in the lungs during the early stages following the injection of brucella. From these changes there is noticed a transition to the development of granulomata which are filled with venous blood; other granulomata disseminate the periphery in the thickened interalveolar partitions.

Degeneration in the lungs also begins in the early stages following the injection of tubercle bacilli, with proliferation of local connective tissue cells in the adventitia of small veins and walls of the alveolus. In the friable connective tissue surrounding the branches of the small bronchi there is also noticed an infiltration of mononuclear cells. Leukocytes and singular epithelioid cells appear in these cellular accumulations in a considerable quantity after 10 days. The quantity of the latter progressively increases, and after approximately 3 weeks many epithelioid cellular tubercles are observed in the lungs. General contours of the alveolus are seen in the foci of some of them. Others are either filled with blood from small veins or small bronchi. Some tubercles on the periphery disseminate into the thickened wall of the pulmonary alveoli which contain epithelioid cells. Singular giant cells appear among the elements of the granulation tissue after a month. The focus of the granulation tissue is subjected to caseous necrosis. A few tubercle bacilli are observed in tubercular granulomata of

the lungs, as well as in other organs. The quantity of connective tissue fibers in the intercellular elements of the granulation tissue gradually increases. The tubercular nidi increase in size considerably, with serous liquid and leukocytes appearing in the caseous masses after 2½-3 months. The necrotic masses become soft, and a strip is formed in the center of the nidi, the walls of which are covered with masses of caseous degeneration. The latter contains an enormous amount of bacilli.

Together with epithelioid cellular nidi, changes of a more diffused character are also observed in the tissues of the lungs. The pulmonary alveoli are filled in places with macrophages and singular giant cells. Later nidi of caseous necrosis are also observed in such sections.

Small blotches with nidi formed by interwoven partially hyaline collagenous fibers can be seen in places under the pleura. Small complexes of epithelioid cells or nidi of caseous necrosis are sometimes seen among them.

In singular cases of experimental brucellosis, nidus accumulations of epithelioid and giant cells appear in the interstitial tissue of the spermatic glands. Polyblasts and a large quantity of plasmatic cells are seen among them. In such cases the natural coverings of the separate ducts are destroyed, and their epithelium is directly meshed with granulation tissue.

Specific changes in the spermatic glands also take place only in singular tuberculosis cases. Nidi of caseous necrosis surrounded with epithelioid and singular giant cells appear in the spermatic glands. However, in contrast to brucellosis, a large quantity of connective tissue fibers are formed between the cellular elements of the granulation tissue. In those cases where a fusion of the granulation tissue and the capsule of the spermatic gland takes place, the capsule becomes greatly thickened.

Conclusions

1. Morphological changes observed in experimental tuberculosis and brucellosis in guinea pigs are different; these diseases can be differentiated even by a single macroscopic study.

2. At the site of the cultural injection an abscess which ruptures exteriorly is formed in tuberculosis, and in its place an obstinate ulcer remains. No change arises in brucellosis in the site of the cultural injection.

3. No macroscopic changes are observed in the internal organs in the case of general brucellosis. On the other hand, in general tuberculosis many miliary tubercles are seen in the lungs, liver, and spleen. In later stages, primary changes begin in the liver and spleen. Many large yellow-white necrotic nidi first appear in these organs; cavities are formed in the lungs after softening of the larger caseous nidi.

4. Microscopic changes in the first 10-14 days after injection have some similarity in both diseases. For example, in the lymphatic nodules, both regional and singular, as well as in the spleen, a progressive hyperplasia of the reticulo-endothelial cells gradually develops. Hypertrophy and hyperplasia of the Kupffer's cells are observed in the liver, as well as proliferation of the connective tissue elements in the adventitia of the small veins, which leads to the formation of many granulomata. Granulomata are also formed in the lungs as a result of the proliferation of the connective tissue cells in the adventitia of the veins near the

bronchi and in the interalveolar partitions.

5. After the 10th day significant differences in the character and course of morphological changes taking place in both infections begin to appear.

6. In brucellosis the many granulomata which are formed in the lungs and liver do not have a tendency to increase further. Specific granulation tissue grows diffusely only in the periportal layers in brucellosis. On the other hand, granulomata in both the lungs and the liver grow very rapidly in tuberculosis and their foci undergo caseous necrosis.

7. A diffused hyperplasia of the cells of the reticulo-endothelial system develops in the spleen in brucellosis. In tuberculosis, hyperplasia of the same elements bears a predominantly nidal character.

8. In contrast to other organs, morphological changes develop much earlier and more acutely in separate lymphatic nodules in experimental brucellosis than in general tuberculosis. Degeneration in tuberculosis is observed here usually not earlier than 3 weeks after injection.

9. A caseous necrosis of specific granulation tissue is always observed in experimental tuberculosis. In brucellosis, necrosis of the granulation tissue is noticed only in singular cases in regional lymphatic nodules.

10. A development of connective tissue fibers is never observed in brucella granulomata. On the other hand, fibrous stroma is formed very early among the cells of the granulation tissue and reaches an acute degree of development in tuberculosis. Argyrophilic fibers formed among the cells of tubercular granulation tissue collagenize very quickly. In later stages, an incapsulation and formation of large caseous nidi in the liver and spleen and the formation of a connective tissue wall in the pulmonary cavities are observed.

11. In connection with an acute new formation of connective tissue fibers in the liver, extensive cirrhotic changes develop in tuberculosis after approximately 2½ months. (See also the works of Pagel, Stoerk, Catsaras.) Anemic infarcts are formed in the liver and the spleen in conjunction with affection of the vessels in tuberculosis. On the other hand, in experimental brucellosis neither cirrhotic changes nor affection of the vessels is observed. An acute toxic inflammation of the liver (the so-called acute serous hepatitis of Roessle and Eppinger) was noticed only in singular cases of experimental brucellosis, and it was not observed in tuberculosis.

12. Literary data indicating a similarity of morphological changes in experimental tuberculosis and brucellosis in guinea pigs (Smith and Fabian, Klimmer and Haupt, Lotze, Seifarth) was not completely confirmed in our studies. Nonspecific microscopic changes, showing some similarity in both infections, develop only in the early stages following the injection. However, the changes which develop in the later periods make it possible to differentiate these diseases very easily.

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